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(54) Title: USE OF INTERFERON-INDUCIBLE 2',5'-OLIGOADENYLATE-DEPENDENT RNase L IN THE DIAGNOSIS, PROGNOSIS AND TREATMENT OF CANCER

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(57) Abstract: A method of detecting cancer or a predisposition to cancer in a mammal, which method comprises detecting at least one mutation in a gene encoding interferon-inducible 2',5'-oligoadenylate-dependent RNase L (RNase L) in a test sample comprising a nucleic acid comprising the RNase L gene obtained from the mammal; a method of determining the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene in a test sample comprising a nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene obtained from a mammal, which method comprises assaying the test sample for the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene; a method for detecting cancer or a predisposition to cancer in a mammal, which method comprises detecting a mutant RNase L in a test sample comprising protein comprising RNase L obtained from the mammal; a method of determining the level of wild-type RNase L and/or a mutant RNase L in a test sample comprising protein comprising wild-type RNase L and/or a mutant RNase L obtained from a mammal, which method comprises assaying the test sample for the level of wild-type RNase L and/or a mutant RNase L; and a method of treating a mammal prophylactically or therapeutically for cancer, wherein the cancer is due to a complete or partial loss of wild-type RNase L, which method comprise providing RNase L to the mammal.

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USE OF INTERFERON-INDUCIBLE 2',5'-OLIGOADENYLATE-DEPENDENT RNase L IN THE DIAGNOSIS, PROGNOSIS AND TREATMENT OF CANCER

FIELD OF THE INVENTION

[0001] This invention pertains to the use of interferon-inducible 2',5'-oligoadenylate-dependent RNase L in the diagnosis, prognosis and treatment of cancer, particularly prostate cancer.

BACKGROUND OF THE INVENTION

[0002] Prostate cancer is the most common non-cutaneous malignancy diagnosed in men in the United States, accounting for over 40,000 deaths annually (Parker *et al.*, *J. Clin. Cancer.* 46:5, 1996). While methods for early detection and treatment of prostate cancer have been forthcoming, there is an obvious need for improvement in this area. Therefore, the discovery of gene mutations which are good indicators of cancer, and more particularly prostate cancer, would be a tremendous step towards understanding the mechanisms underlying cancer and could offer a dramatic improvement in the ability of scientists to detect cancer and even to predict an individual's susceptibility to a particular type of cancer.

[0003] Much research has, in fact, been centered around establishing a genetic link to prostate cancer and studies have identified many recurring genetic changes associated with prostate cancer. These genetic changes include DNA hypermethylation, allelic loss, aneuploidy, aneusomy, various point mutations, and changes in protein expression level (*e.g.*, E-cadherin/alpha-catenin). Researchers have also discovered losses and duplications in particular chromosomes or chromosome arms which are associated with prostate cancer (U.S. Patent No. 5,925,519; Visakorpi, *Ann. Chirur. Gynaec.* 88:11-16, 1999). In particular, losses of chromosomes 6q, 8p, 10q, 13q and 16q, and duplications of 7, 8q and Xq have been associated with prostate cancer. Moreover, researchers have performed genetic epidemiological studies of affected populations and have identified various putative prostate cancer susceptibility loci, indicating that there is significant genetic heterogeneity in prostate cancer. These include Xq27-q28 (Xu *et al.*, *Nat. Genet.* 20:175-179, 1998) and 1q42-q43 (Gibbs *et al.*, *Am. J. Hum. Genet.* 64:1087-1095; Berthon *et al.*, *Am. J. Hum. Genet.* 62:1416-1424, 1998).

[0004] One such potential prostate cancer susceptibility locus is the 1q24-q31 locus (flanked by D1S2883 and D1S422), which has been designated as HPC1 (due to its putative link to hereditary prostate cancer (HPC)). This HPC1 locus was identified by the inventors in a genome-wide scan of families at high risk for prostate cancer (Smith *et al.*, *Science* 274:1371-1374, 1996). The HPC1 locus has been controversial, however, due to the fact

that researchers have had difficulty duplicating the results of Smith et al. (De la Chapelle and Peltomaki, *Curr. Opin. Genet. Dev.* 8:298-303, 1998). In fact, some groups of researchers have found no linkage of the HPC1 locus to hereditary prostate cancer (Eeles *et al.*, *Am. J. Hum. Genet.* 62:653-658, 1998; Thibodeau *et al.*, *Am. J. Hum. Genet.* 61(suppl):1733, 1997; McIndoe *et al.*, *Am. J. Hum. Genet.* 61:347-353, 1997), while others have found linkage in a very small fraction of high-risk prostate cancer families (Schleutker *et al.*, *Am. J. Hum. Genet.* 61(suppl):1711, 1997). Further support for the linkage between the HPC1 locus and hereditary prostate cancer was revealed, however, via a combined Consortium analysis of 6 markers in the HPC1 region in 772 families segregating hereditary prostate cancer (see Xu *et al.*, *Am J Hum Genet.* 66:945-957, 2000). In this regard, research findings concerning the HPC1 locus and its potential link to prostate cancer have been promising, but often nonconforming.

[0005] Therefore, there remains a need for the identification of genes and gene products which can be shown to have a strong association with prostate cancer. Such genes and gene products can lead directly to early, sensitive and accurate methods for detecting cancer or a predisposition to cancer in a mammal. Moreover, such methods would enable clinicians to monitor the onset and progression of cancer in an individual with greater sensitivity and accuracy, as well as the response of an individual to a particular treatment. The present invention provides such methods.

BRIEF SUMMARY OF THE INVENTION

[0006] A method of detecting cancer or a predisposition to cancer in a mammal is provided. The method comprises detecting at least one mutation in a gene encoding interferon-inducible 2',5'-oligoadenylate-dependent RNase L (RNase L) in a test sample comprising a nucleic acid comprising the RNase L gene obtained from the mammal, wherein the at least one mutation is indicative of cancer or a predisposition to cancer in the mammal.

[0007] Also provided is a method of determining the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene in a test sample comprising a nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene obtained from a mammal. The method comprises assaying the test sample for the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene, wherein a decrease in the level of nucleic acid comprising the wild-type RNase L gene and/or an increase in the level of nucleic acid comprising a mutant RNase L gene in the test sample as compared to a control sample is indicative of prostate cancer or a predisposition to prostate cancer in the mammal.

[0008] Further provided is a method for detecting cancer or a predisposition to cancer in a mammal. The method comprises detecting a mutant RNase L in a test sample comprising protein comprising RNase L obtained from the mammal, wherein the presence of a mutant RNase L in the test sample is indicative of cancer or a predisposition to cancer in the mammal.

[0009] Still further provided is a method of determining the level of wild-type RNase L and/or a mutant RNase L in a test sample comprising protein comprising wild-type RNase L and/or a mutant RNase L obtained from a mammal. The method comprises assaying the test sample for the level of wild-type RNase L and/or a mutant RNase L, wherein a decrease in the level of wild-type RNase L and/or an increase in the level of a mutant RNase L in the test sample as compared to a control sample is indicative of cancer or a predisposition to cancer in the mammal.

[0010] The invention further provides a method of treating a mammal prophylactically or therapeutically for cancer, wherein the cancer is due to a complete or partial loss of wild-type RNase L, which method comprises providing RNase L to the mammal, whereupon the mammal is treated prophylactically or therapeutically for cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Figure 1A is a graph of nucleic acid position versus the likelihood of odds (LOD), which represents a multi-point linkage analysis performed under the assumption of heterogeneity on 91 high-risk prostate cancer families, and which demonstrates that an HPC1 gene maps to the interval D1S2883-D1S158-D1S422.

[0012] Figure 1B is a table of nucleic acid position versus pedigree, which represents a comparison of affected recombinants.

[0013] Figure 1C is a transcript map of the chromosome 1 locus 1q25, which comprises the RNase L gene.

[0014] Figure 1D is a schematic diagram of the RNase L gene, with the translational start site and the stop site denoted by codons ATG and TGA, respectively.

[0015] Figure 1E is a schematic diagram of the RNase L protein, which illustrates the major domains of the 741 amino acid protein.

[0016] Figure 2A is a schematic diagram which illustrates the pedigree structure, a sequenced-based mutational analysis chromatogram, and a loss of heterozygosity (LOH) analysis for high-risk HPC1 family 065. Variable nucleotides are marked by a dot in each representative chromatogram and wild-type and mutant alleles of RNase L are marked by arrows.

[0017] Figure 2B is a schematic diagram which illustrates the pedigree structure, a sequenced-based mutational analysis chromatogram, and an LOH analysis for high-risk

HPC1 family 097. Variable nucleotides are marked by a dot in each representative chromatogram and wild-type and mutant alleles of RNase L are marked by arrows.

DETAILED DESCRIPTION OF THE INVENTION

[0018] The invention provides a method for detecting cancer or a predisposition to cancer (i.e., carcinoma) in a mammal. The method comprises detecting at least one mutation in a gene encoding RNase L in a test sample comprising a nucleic acid comprising the RNase L gene obtained from the mammal, wherein the at least one mutation is indicative of cancer or a predisposition to cancer in the mammal. "Nucleic acids" and "genes" as used herein can be either deoxyribonucleic acid (DNA) (e.g., cDNA) or ribonucleic acid (RNA) (e.g., mRNA). Moreover, the nucleic acids and genes can comprise exons, introns, and/or regulatory regions and elements.

[0019] The method can be used for detecting any type of cancer or predisposition to cancer in a mammal. It is suitable, for example, for the method to be used for detecting the presence of, or a predisposition to, prostate cancer, testicular cancer, uterine cancer, intestinal cancer, colon cancer, lung cancer, liver cancer, or lymphoid cancer in a mammal. Preferably, the method is used to detect prostate cancer, or a predisposition to prostate cancer, in a mammal. More preferably, the method is used to detect HPC or a predisposition to HPC in a mammal. Most preferably, the method is used to detect HPC1-associated prostate cancer or a predisposition to HPC1-associated prostate cancer in a mammal.

[0020] A mammal is defined herein as any male or female mammal (e.g., human). Moreover, it will be understood that a method for detecting prostate cancer or testicular cancer, or predisposition thereto, can only be performed on male mammals, whereas a method for detecting uterine cancer or a predisposition to uterine cancer can only be performed on female mammals.

[0021] The test sample used in conjunction with the method can be any of those typically used in the art. For example, the test sample can be tissue, such as, for example, metastatic (e.g., cancerous) tissue obtained by means of a biopsy. Such tissue can include bone marrow, lymph nodes, skin, glands, and any organ that may develop cancerous cells. Preferably, the test sample is taken from a source that is typically used and is known in the art to detect prostate cancer (e.g., HPC). Suitable test samples include, for example, urine, semen, lymph, and blood serum.

[0022] The at least one mutation (e.g., at least two mutations, at least three mutations, at least four mutations, at least five mutations, or even at least ten mutations) in a gene encoding RNase L is defined herein as any one or more mutations in the gene encoding RNase L which is/are indicative of cancer or a predisposition to cancer in a mammal. The

at least one mutation can be, for example, any frame-shift mutations, missense mutations and/or nonsense mutations, arising from any insertion, duplication, deletion, and/or substitution in a gene encoding RNase L. The at least one mutation can cause transcriptional, post-transcriptional, translational, and/or post-translational processing errors, e.g., a translation error wherein translation begins at a codon encoding a methionine other than the first methionine of the RNase L gene (e.g., a codon encoding the third methionine of the RNase L gene). Moreover, the at least one mutation in the RNase L gene can cause one or more splicing errors (i.e., splicing mutations), such that a mutant RNase L gene is produced. Alternatively, or in addition to, the at least one mutation in the RNase L gene can be a mutation that causes transcriptional, post-transcriptional, translational, and/or post-translational processing of the RNase L gene to stop prematurely, thereby leading to the expression of a truncated form of RNase L. The at least one mutation can also cause a decreased efficiency of transcriptional, post-transcriptional, translational, and/or post-translational processing of the RNase L gene product. Moreover, the at least one mutation in the RNase L gene can be associated with a compromised ability of the RNase L gene product to function normally, as compared to wild-type RNase L.

[0023] The at least one mutation in the RNase L gene is indicative of cancer or a predisposition to cancer in the mammal if, for example, the at least one mutation compromises the tumor-suppressing activity of the RNase L gene product, as compared to normal (i.e., wild-type) RNase L. The at least one mutation in the RNase L gene also is indicative of cancer or a predisposition to cancer in a mammal if the at least one mutation compromises the ability of the RNase L gene product to interact with (i.e., bind to) 2'-5'-oligoadenylate, as compared to wild-type RNase L. Moreover, the at least one mutation in the RNase L gene is indicative of cancer or a predisposition to cancer in a mammal if the at least one mutation compromises the ability of the RNase L gene product to become activated, as compared to wild-type RNase L; compromises the ability of the RNase L gene product to dimerize, as compared to wild-type RNase L; compromises the ability of the RNase L gene product to degrade double-stranded RNA, as compared to wild-type RNase L; and/or compromises the ability of the RNase L gene product to induce apoptosis (e.g., to induce apoptosis through the 2-5A pathway), as compared to wild-type RNase L. In this regard, the wild-type functional capacity of RNase L to become active, to dimerize, to degrade RNA, and to act in the 2-5A apoptotic pathway is believed to be dependent on the interaction between RNase L and 2'-5'-oligoadenylate. Moreover, in cells of mammals possessing only one non-mutated (i.e., wild-type) allele for the RNase L gene, the at least one mutation in the RNase L gene can result in a loss of such heterozygosity (LOH).

[0024] The at least one mutation in the RNase L gene can be detected at one or more nucleic acid positions of the RNase L gene, e.g., within any exon, intron, and/or regulatory

region of the RNase L gene. It is suitable, for example, for the at least one mutation of the RNase L gene to be detected in intron 1, intron 2, intron 3, intron 4, intron 5, or even intron 6 of the RNase L gene. Moreover, it is suitable, for example, for the at least one mutation of the RNase L gene to be detected in exon 1 (e.g., in the transcriptional start site of exon 1), exon 2, exon 3, exon 4, exon 5, exon 6, or even exon 7 of the RNase L gene. The second exon of the RNase L gene is defined herein as that portion of the RNase L gene which encodes for and expresses the binding site for 2',5'-oligoadenylate and which also is involved in protein-protein interactions. Alternatively, or in addition to, the at least one mutation also can be detected outside of the second exon of the RNase L gene, wherein the at least one mutation affects the transcription, post-transcriptional processing, translation, and/or post-translational processing of the second exon of the RNase L gene. For example, at least one mutation of the gene encoding RNase L can be detected at position 795 of the second exon of the RNase L gene, e.g., a guanine (G) to thymine (T) substitution at position 795 of the second exon of the RNase L gene, in the initiation codon of the RNase L gene, e.g., at position 3 of the second exon of the RNase L gene, such as an adenine (A) to guanine (G) substitution at position 3 of the second exon. Alternatively, or in addition to, the at least one mutation of the gene encoding RNase L can be detected at position 175 of the second exon of the RNase L gene, e.g., a G to A substitution at position 175 of the second exon. In this regard, mutations can be detected at position 175 of the second exon of the RNase L gene, at position 795 of the second exon of the RNase L gene, and in the initiation codon of the RNase L gene, e.g., to detect a G to A substitution at position 175 of the second exon, a G to T substitution at position 795 of the second exon, and a mutation at position 3 of the initiation codon of the second exon e.g., an A to G substitution.

[0025] The RNase L gene in a test sample obtained from a mammal can be amplified using any suitable amplification method known in the art, e.g., polymerase chain reaction (PCR); reverse transcriptase PCR (RT-PCR); ligase chain reaction (LCR) (disclosed in U.S. Patent No. 4,883,750); isothermal amplification (disclosed in Walker et al., *Proc. Natl Acad. Sci. USA* 89: 392-396 (1992)); strand displacement amplification (SDA); and repair chain reaction (RCR). Target-specific sequences also can be detected using a cyclic probe reaction (CPR). Moreover, alternative methods for reverse transcription are described in WO 90/07641.

[0026] Any primer sequences can be used in the amplification process, as long as the primer sequences are hybridizable to nucleic acids encoding a wild-type RNase L gene, a mutant RNase L gene, and/or functional sequence analogs thereof. For example, M13-tailed primers can be used in the amplification process (see Table 1).

[0027] The nucleic acid used as a template for amplification can be isolated from a test sample using any standard methodology (Sambrook et al., *Molecular Cloning: A Laboratory*

Manual, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1989).

Alternatively, or in addition to, chromatographic techniques can be employed to effect separation. It will be understood that there are many kinds of chromatography which can be used in the context of the method, e.g., adsorption, partition, ion-exchange and molecular sieve, and many specialized techniques for using them including column, paper, thin-layer and gas chromatography (Freifelder, *Physical Biochemistry Applications to Biochemistry and Molecular Biology*, 2nd Ed., Wm. Freeman and Co., New York, N.Y. (1982)).

[0028] Amplification products must be visualized in order to confirm amplification of the RNase L gene. One typical visualization method involves staining of a gel with ethidium bromide and visualization under UV light. Alternatively, if the amplification products are integrally labeled with radio- or fluorometrically-labeled nucleotides, the amplification products can then be exposed to x-ray film or visualized under the appropriate stimulating spectra, following separation. In one embodiment, visualization is achieved indirectly. Following separation of amplification products, a labeled, nucleic acid probe is brought into contact with and allowed to hybridize with the amplified RNase L gene sequence. The probe preferably is conjugated to a chromophore, but may be radiolabeled. In another embodiment, the probe is conjugated to a binding partner, such as an antibody or biotin, where the other member of the binding pair carries a detectable moiety (i.e., a label). One example of the foregoing is described in U.S. Patent No. 5,279,721, which discloses an apparatus and method for the automated electrophoresis and transfer of nucleic acids.

[0029] When hybridization is employed, preferably, the hybridization is done under high stringency conditions. By "high stringency conditions" is meant that the probe specifically hybridizes to a target sequence in an amount that is detectably stronger than non-specific hybridization. High stringency conditions, then, are conditions that distinguish a polynucleotide with an exact complementary sequence, or one containing only a few scattered mismatches from a random sequence that happened to have a few small regions (e.g., 3-10 bases) that matched the probe. Such small regions of complementarity are more easily melted than a full-length complement of 14-17 or more bases, and high stringency hybridization makes them easily distinguishable. Relatively high stringency conditions include, for example, low salt and/or high temperature conditions, such as provided by about 0.02-0.1 M NaCl or the equivalent, at temperatures of about 50-70 °C. Such relatively high stringency conditions tolerate little, if any, mismatch between the probe and the template or target strand, and are particularly suitable for detecting expression of specific RNase Ls. It is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide.

[0030] The at least one mutation in the RNase L gene also can be detected by sequencing. For example, an exon of the amplified RNase L gene can be sequenced using

techniques known in the art and compared to the sequence of the corresponding exon of a control RNase L gene, using computer software. A suitable control RNase L gene sequence for use in the method is, for example, a publicly available RNase L gene (Online Mendelian Inheritance in Man™ (OMIM) #180435) (Johns Hopkins University, Baltimore, MD).

[0031] In addition to the above, the invention provides a method of determining the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene in a test sample comprising a nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene obtained from a mammal. The method comprises assaying the test sample for the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene, wherein a decrease in the level of nucleic acid comprising the wild-type RNase L gene and/or an increase in the level of nucleic acid comprising a mutant RNase L gene in the test sample as compared to a control sample is indicative of cancer (e.g., prostate cancer) or a predisposition to cancer in the mammal.

[0032] A wild-type RNase L gene is defined herein as any RNase L gene that encodes an RNase L gene product that has (i.e., possesses) tumor-suppressing activity, the ability to bind to 2'-5'-oligoadenylate, the ability to become activated, the ability to dimerize, the ability to degrade double-stranded RNA, and the ability to induce apoptosis (e.g., to induce apoptosis through the 2-5A pathway). A mutant RNase L gene is defined herein as any RNase L gene that encodes an RNase L gene product which has a compromised ability (i.e., little or no ability) to act as a tumor suppressor, as compared to wild-type RNase L; a compromised ability to bind to 2'-5'-oligoadenylate, as compared to wild-type RNase L; a compromised ability to become activated, as compared to wild-type RNase L; a compromised ability to dimerize, as compared to wild-type RNase L; a compromised ability to degrade double-stranded RNA, as compared to wild-type RNase L; and/or a compromised ability to induce apoptosis (e.g., to induce apoptosis through the 2-5A pathway), as compared to wild-type RNase L. As mentioned previously, the wild-type functional capacity of RNase L to become active, to dimerize, to degrade RNA, and to act in the 2-5A apoptotic pathway is believed to be dependent on the interaction between RNase L and 2'-5'-oligoadenylate. Moreover, the binding site for 2'-5'-oligoadenylate within RNase L is believed to be encoded by the second exon of the RNase L gene.

[0033] The level of a wild-type RNase L gene and/or a mutant RNase L gene in a test sample obtained from a mammal is defined herein as the quantity of nucleic acid comprising a wild-type RNase L gene and/or the quantity of nucleic acid comprising a mutant RNase L gene in the test sample. "Decreased" and "increased" levels of the wild-type RNase L gene and/or a mutant RNase L gene are determined by a comparison of the level of wild-type and/or mutant RNase L genes present in a test sample obtained from a mammal to any suitable control test sample. Suitable control test samples include, for

example, a test sample obtained from the same mammal at a different point in time and a test sample obtained from a different mammal of the same species (e.g., a mammal of the same sex which has been confirmed to cancer-free).

[0034] Various assays can be used to measure the presence and/or level of nucleic acid (i.e., DNA or RNA) comprising a wild-type RNase L gene and/or a mutant RNase L gene present in a test sample obtained from a mammal. For example, assays including PCR and microarray analysis can be used to detect the presence and/or absence of the wild-type RNase L gene and/or a mutant RNase L gene, as described, for example, in U.S. Patent Nos. 6,197,506 and 6,040,138. Moreover, it is understood that the type of assay used depends on whether the nucleic acid of interest being assayed is DNA or RNA. Assays for determining the level of DNA comprising a wild-type RNase L gene and/or a mutant RNase L gene in a test sample include, for example, Southern hybridization (i.e., a Southern blot), *in situ* hybridization and microarray analysis. Assays for determining the level of RNA (e.g., mRNA) comprising a wild-type RNase L gene and/or a mutant RNase L gene in a test sample include, for example, Northern hybridization (i.e., a Northern blot), *in situ* hybridization and microarray analysis.

[0035] It is also understood that a nucleic acid sequence that specifically binds to, or associates with, a nucleic acid comprising a gene encoding RNase L, whether DNA or RNA, can be attached to a label for determining hybridization. A wide variety of appropriate labels are known in the art, including, for example, fluorescent, radioactive, and enzymatic labels, as well as ligands (e.g., avidin/biotin), which are capable of being detected. Preferably, a fluorescent label or an enzyme tag, such as urease, alkaline phosphatase or peroxidase, is used instead of a radioactive or other environmentally undesirable label. In the case of enzyme tags, colorimetric indicator substrates are known which can be employed to provide a detection system that is visible spectrophotometrically, or even visible to the human eye to identify specific hybridization with complementary RNase L nucleic acid-containing samples.

[0036] The invention also provides a method for prognosticating a cancer (e.g., prostate cancer) in a mammal. The method comprises determining the level of nucleic acid comprising the wild-type RNase gene and/or a mutant RNase L gene in a test sample comprising a nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene obtained from the mammal, and comparing the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene in the test sample to the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene, respectively, in another test sample obtained from the mammal over time, wherein a decrease in the level of nucleic acid comprising the wild-type RNase L gene and/or an increase in the level of nucleic acid comprising a mutant RNase L gene is indicative of an

unfavorable prognosis, an increase in the level of the nucleic acid comprising the wild-type RNase L gene and/or a decrease in the level of the nucleic acid comprising a mutant RNase L gene is indicative of a favorable prognosis, and no change in the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene is indicative of no change in the cancer.

[0037] The invention also provides a method for assessing the efficacy of treatment of a cancer (e.g., prostate cancer) in the mammal with a given anti-cancer agent. The method comprises determining the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene in a test sample comprising a nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene obtained from the mammal, and comparing the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene in the test sample to the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene, respectively, in another test sample obtained from the mammal over time, wherein a decrease in the level of nucleic acid comprising the wild-type RNase L gene and/or an increase in the level of nucleic acid comprising a mutant RNase L gene is indicative of the anti-cancer agent being effective, an increase in the level of the nucleic acid comprising the wild-type RNase L gene and/or a decrease in the level of the nucleic acid comprising a mutant RNase L gene is indicative of the anti-cancer agent being ineffective, and no change in the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene is indicative of no change in the cancer due to treatment with the anti-cancer agent.

[0038] A mutant RNase L gene product also can be detected in a test sample obtained from a mammal and is indicative of cancer or a predisposition to cancer in the mammal. Accordingly, the present invention further provides a method for detecting cancer or a predisposition to cancer in a mammal. The method comprises detecting a mutant RNase L in a test sample comprising protein comprising RNase L obtained from the mammal, wherein the presence of a mutant RNase L in the test sample is indicative of cancer or a predisposition to cancer in the mammal. For example, the method can comprise detecting a mutant RNase L in a test sample comprising protein comprising RNase L obtained from the mammal, wherein the tumor-suppressing activity of the mutant RNase L is compromised. Moreover, the method can comprise detecting a mutant RNase L in a test sample comprising protein comprising RNase L obtained from the mammal, wherein the ability of the mutant RNase L to bind to 2'-5'-oligoadenylate, to degrade double-stranded RNA, and/or to induce apoptosis is compromised as compared to wild-type RNase L. The determination that the ability of an RNase L to act as a tumor suppressor, to bind to 2'-5'-oligoadenylate, to degrade double-stranded RNA, and/or to induce apoptosis is compromised as compared to wild-type RNase L can be done in accordance with methods

known in the art. The method also can comprise detecting at least one mutation in any domain of a mutant RNase L in a test sample comprising protein, e.g., detecting at least one mutation in one or more of the nine ankyrin-like repeats of RNase L, detecting at least one mutation in the phosphate binding loop (p-loop) region of RNase L, detecting at least one mutation in the tyrosine kinase-like domain at the carboxy terminus of RNase L, and/or detecting at least one mutation in the ribonuclease domain of RNase L also in accordance with methods known in the art. For example, the method can comprise detecting a mutation at amino acid position 265 of a mutant RNase L in a test sample comprising protein comprising RNase L obtained from the mammal, e.g., wherein the mutation at amino acid position 265 of the mutant RNase L results in the conversion of a glutamic acid codon to a termination codon. Moreover, the method can comprise detecting a mutation at amino acid position 1 of a mutant RNase L in a test sample comprising protein comprising RNase L obtained from the mammal, e.g., wherein the mutation at amino acid position 1 of the mutant RNase L results in the conversion of a methionine codon to an isoleucine codon.

[0039] Alternatively, the levels of wild-type RNase L and/or a mutant RNase L can be determined. Accordingly, the invention also provides a method of determining the level of wild-type RNase L and/or a mutant RNase L in a test sample comprising protein comprising wild-type RNase L and/or a mutant RNase L obtained from a mammal. The method comprises assaying the test sample for the level of wild-type RNase L and/or a mutant RNase L, wherein a decrease in the level of wild-type RNase L and/or an increase in the level of a mutant RNase L in the test sample as compared to a control sample (as described previously) is indicative of cancer or a predisposition to cancer in the mammal.

[0040] Various assays (i.e., immunobinding assays) are contemplated for detecting and/or measuring the quantity of wild-type RNase L and/or a mutant RNase L in a test sample obtained from a mammal. For example, separate and distinct antibodies can be prepared and employed to detect wild-type RNase L and a mutant RNase L, respectively. Alternatively, wild-type RNase L and a mutant RNase L can be utilized to detect antibodies having reactivity therewith. The steps of various useful immunodetection assays have been described, for example, in Nakamura et al., *Handbook of Experimental Immunology* (4th Ed.), Vol. 1, Chapter 27, Blackwell Scientific Publ., Oxford (1987); Nakamura et al., *Enzyme Immunoassays: Heterogenous and Homogenous Systems*, Chapter 27 (1987). Suitable immunoassays include, for example, Western hybridization (i.e., Western blots), immunoaffinity purification, immunoaffinity detection, enzyme-linked immunosorbent assay (e.g., an ELISA), and radioimmunoassay. Moreover, a microarray can be used to detect and/or measure the levels of wild-type RNase L and/or a mutant RNase L in a test sample obtained from a mammal.

[0041] In general, the immunobinding assays involve obtaining a test sample suspected of containing a protein, peptide, polypeptide, and/or antibody corresponding to wild-type RNase L and/or a mutant RNase L, and contacting the test sample with one or more antibodies under conditions effective to allow the formation of immunocomplexes. It is suitable, for example, to contact concurrently, or sequentially, a test sample obtained from a mammal with an antibody that is specific to wild-type RNase L and with an antibody that is specific to a mutant RNase L.

[0042] Any suitable antibody can be used in conjunction with the present invention such that the antibody is specific for wild-type RNase L. Likewise, any suitable antibody can be used in conjunction with the present invention such that the antibody is specific for a mutant RNase L. In particular, suitable antibodies recognize and interact with (i.e., bind to) one or more portions of wild-type RNase L and with one or more portions of a mutant RNase L. Moreover, suitable antibodies include antibodies that recognize and interact with other antibodies present in a test sample that bind to wild-type RNase L. Likewise, suitable antibodies include antibodies that recognize and interact with other antibodies present in a test sample that bind to a mutant RNase L. Antibodies for use in the present inventive methods can be produced by any known technique, e.g., as described in *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, (1988)).

[0043] Contacting a test sample comprising a protein comprising wild-type RNase L and/or a mutant RNase L with an antibody or antibodies that recognize wild-type RNase L and/or a mutant RNase L under conditions effective, and for a period of time sufficient, to allow for formation of immune complexes (primary immune complexes) is generally a matter of adding the antibody to the test sample and incubating the mixture for a period of time long enough for the antibodies to form immune complexes with wild-type RNase L and/or a mutant RNase L. Detection of immunocomplex formations can be achieved through the application of numerous techniques which are well-known in the art. These detection methods are generally based upon the detection of a label or marker, such as any radioactive, fluorescent, biological and enzymatic labels of standard use in the art, as described, for example, in U.S. Patents Nos. 3,817,837, 3,850,752, 3,939,350, 3,996,345, 4,277,437, 4,275,149 and 4,366,241. Of course, additional advantages can be realized by using a secondary binding ligand, such as a second antibody or a biotin/avidin ligand binding arrangement, as is known in the art.

[0044] The antibody or antibodies which is/are used in the context of the present invention can, themselves, be linked to a detectable label. Such a detectable label allows for the presence of, or the amount of, the primary immune complexes to be determined. Alternatively, the first added component that becomes bound within the primary immune complexes can be detected by means of a second binding ligand that has binding affinity for

the first antibody. In these cases, the second binding ligand is, itself, often an antibody, which can be termed a "secondary" antibody. The primary immune complexes are contacted with the labeled, secondary binding ligand, or antibody, under conditions effective and for a period of time sufficient to allow the formation of secondary immune complexes. The secondary immune complexes are then washed to remove any non-specifically bound labeled secondary antibodies or ligands, and the remaining label in the secondary immune complexes is then detected.

[0045] Further methods include the detection of primary immune complexes by a two-step approach. A second binding ligand, such as an antibody, that has binding affinity for the first antibody is used to form secondary immune complexes, as described above. After washing, the secondary immune complexes are contacted with a third binding ligand or antibody that has binding affinity for the second antibody, again under conditions effective and for a period of time sufficient to allow the formation of immune complexes (tertiary immune complexes). The third ligand or antibody is linked to a detectable label, allowing detection of the tertiary immune complexes thus formed.

[0046] The invention also provides a method for prognosticating a cancer in a mammal. The method comprises comparing the level of wild-type RNase L and/or a mutant RNase L in the test sample to the level of wild-type RNase L and/or a mutant RNase L, respectively, in another test sample obtained from the mammal over time, wherein a decrease in the level of wild-type RNase L and/or an increase in the level of a mutant RNase L is indicative of an unfavorable prognosis, an increase in the level of the wild-type RNase L and/or a decrease in the level of a mutant RNase L is indicative of a favorable prognosis, and no change in the level of the wild-type RNase L and/or a mutant RNase L gene is indicative of no change in the cancer.

[0047] The invention also provides a method for assessing the effectiveness of treatment of a cancer in a mammal. The method comprises comparing the level of wild-type RNase L and/or a mutant RNase L in the test sample to the level of wild-type RNase L and/or a mutant RNase L, respectively, in another test sample obtained from the mammal over time, wherein a decrease in the level of the wild-type RNase L and/or an increase in the level of a mutant RNase L is indicative of the anti-cancer agent being effective, an increase in the level of the wild-type RNase L and/or a decrease in the level of a mutant RNase L is indicative of the anti-cancer agent being ineffective, and no change in the level of the wild-type RNase L and/or a mutant RNase L is indicative of no change in the cancer due to treatment with the anti-cancer agent.

[0048] The invention also provides a method of treating a mammal prophylactically or therapeutically for cancer (e.g., prostate cancer), wherein the cancer is due to a complete or

partial loss of wild-type RNase L, which method comprises providing RNase L to the mammal, whereupon the mammal is treated prophylactically or therapeutically for cancer.

[0049] Any suitable method can be used for administering or providing RNase L to a mammal, wherein the RNase L enters the nucleus and/or cytoplasm of one or more cancer cells (e.g., one or more prostate cancer cells) of the mammal and functions within the cell(s) in a manner which is typical of wild-type RNase L. For example, RNase L can be provided to the mammal by administering to the mammal the wild-type RNase L protein, or a portion thereof (e.g., two or more different forms of wild-type RNase L). Moreover, RNase L can be provided to a mammal through administration of a fusion protein comprising wild-type RNase L, or a portion thereof, operably linked to one or more moieties of interest (e.g., two or more, three or more, four or more, or five or more therapeutic moieties, such as tumor suppressor proteins, apoptotic agents, anti-cancer agents, and/or any compounds which potentiate RNase L). In another embodiment, RNase L is provided to a mammal through administration of a nucleic acid encoding and expressing wild-type RNase L, or a portion thereof. Moreover, RNase L can be provided to a mammal through administration of a nucleic acid encoding and expressing a fusion protein comprising wild-type RNase L, or a portion thereof, operably linked to one or more moieties of interest. The administered nucleic acid can be in any suitable form. For example, the administered nucleic acid can be naked DNA or RNA. Moreover, the administered nucleic acid can be part of any suitable vector or vector system. Suitable vectors for use in the method include, for example, plasmid vectors, retroviral vectors, adenoviral vectors, adeno-associated viral vectors, vaccinia virus, sindbis virus, cytomegalovirus, herpes simplex virus, defective hepatitis B viruses, and any other vector or vector system known in the art. Fusion proteins and nucleic acids encoding and expressing fusion proteins can be produced using any standard methods of recombinant production and synthesis known in the art, as described, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1989.

[0050] In view of the above, also provided is a composition. The composition comprises (i) a pharmaceutically acceptable carrier and (ii) RNase L or a portion thereof; a fusion protein comprising RNase L or a portion thereof, operably linked to one or more moieties of interest; a nucleic acid encoding and expressing RNase L or a portion thereof; and/or a nucleic acid encoding and expressing a fusion protein comprising RNase L or a portion thereof, operably linked to one or more moieties of interest.

[0051] The composition can further comprise one or more active ingredients, e.g., pharmaceutically active agents or drugs, anti-cancer agents, and/or any compounds which potentiate RNase L. Suitable anti-cancer agents include, but are not limited to, those anti-cancer agents approved for marketing in the United States and those that will become

approved in the future. See, for example, Table 1 and Table 2 of Boyd, *Current Therapy in Oncology*, Section I. Introduction to Cancer Therapy (J.E. Niederhuber, ed.), Chapter 2, by B.C. Decker, Inc., Philadelphia, 1993, pp. 11-22. More particularly, anti-cancer agents include, for example, doxorubicin, bleomycin, vincristine, vinblastine, VP-16, VW-26, cisplatin, carboplatin, procarbazine, and taxol for solid tumors in general; alkylating agents, such as BCNU, CCNU, methyl-CCNU and DTIC, for brain or kidney cancers; and antimetabolites such as 5-FU and methotrexate for colon cancer.

[0052] The carrier can be any suitable carrier. Preferably, the carrier is a pharmaceutically acceptable carrier. With respect to compositions, the carrier can be any of those conventionally used and is limited only by chemico-physical considerations, such as solubility and lack of reactivity with RNase L, and by the route of administration. It will be appreciated by one of skill in the art that, in addition to the above-described composition, the compositions of the present inventive methods can be formulated as inclusion complexes, such as cyclodextrin inclusion complexes, or liposomes. The pharmaceutically acceptable carriers described herein, for example, vehicles, adjuvants, excipients, and diluents, are well-known to those skilled in the art and are readily available to the public. It is preferred that the pharmaceutically acceptable carrier be one which is chemically inert to RNase L and one which has no detrimental side effects or toxicity under the conditions of use.

[0053] As is understood in the art, the choice of carrier is dependent on several factors, e.g., the type of cancer being treated and the route of administration of the composition. Such a choice of carrier for use in the composition of the present invention is well within the ordinary skill in the art. Accordingly, there are a variety of suitable formulations of the composition of the present invention. Such formulations include but, are not limited to, oral, aerosol, parenteral, subcutaneous, intravenous, intramuscular, interperitoneal, rectal, and vaginal formulations.

[0054] One skilled in the art will appreciate that suitable methods of administering a composition of the invention to a mammal, in particular a human, are available, and, although more than one route can be used to administer a particular compound, a particular route can provide a more immediate and more effective reaction than another route.

[0055] The dose administered to a mammal, in particular a human, should be sufficient to treat the cancer prophylactically therapeutically. One skilled in the art will recognize that dosage will depend upon a variety of factors including the strength of the particular composition employed, as well as the age, species, condition, and body weight of the mammal. The size of the dose will also be determined by the route, timing, and frequency of administration as well as the existence, nature, and extent of any adverse side-effects that

might accompany the administration of a particular composition and the desired physiological effect.

[0056] Suitable doses and dosage regimens can be determined by conventional range-finding techniques known to those of ordinary skill in the art. Generally, a composition is initially administered in smaller dosages, which are less than the optimum dose of the composition. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. The present inventive method will typically involve the administration of about 0.1-100 mg of one or more of the compositions described above per kg body weight.

EXAMPLES

[0057] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

[0058] High-risk prostate cancer families are defined in the following examples as those families comprising at least 4 first-degree relatives sharing an HPC1 haplotype and a likelihood of odds (LOD) score which is greater than 0.7.

[0059] A number of well-known techniques are used in the following examples, including polymerase chain reactions (PCR), DNA sequencing, Northern analysis, laser capture microdissection, fluorescence *in situ* hybridization (FISH), loss of heterozygosity (LOH) analysis, and protein truncation tests (PTT). PCR reactions were performed in a 50 μ l reaction volume, containing 20 ng of genomic DNA, PCR buffer (Gibco BRL), 2.25 mM Mg++, 250 nM dNTPs, 333 nM forward primer, 333 nM reverse primer, 0.6 unit Platinum Taq DNA polymerase (Gibco BRL), 0.06 unit AmpliTaq Gold (PE Biosystems, Foster City, CA), and 10 pmol of the primers (Gibco BRL). PCR cycles consisted of 95°C/14 min, 35 cycles of 95°C/30 s, 56°C/30 s, 72°C/45 s, and a final extension at 72°C/10 min. PCR products were analyzed using 2% agarose gel electrophoresis.

[0060] DNA sequencing and sequence analysis were performed via PCR amplification of the exons of a nucleic acid comprising a wild-type RNase L gene and/or a mutant RNase L gene, using primer sequences derived from the genomic structure of the RNase L gene (i.e., the M13-tailed primers listed in Table 1). PCR products were cleaned using a Qiagen PCR purification kit (Qiagen, Valencia, CA) utilizing the Qiagen BIOROBOT 9600 dual vacuum system. Half cycle sequencing reactions were prepared in 96-well format using the 3700 Big Dye™ Terminator Chemistry (PE/Applied Biosystems, Foster City, CA). Following purification, sequencing reactions were performed, using a 3700 DNA Analyzer (PE/Applied Biosystems, Foster City, CA) and following manufacturer's protocols. Sequence chromatograms were aligned and analyzed using Sequencher version 4.1 (Gene Codes, Ann Arbor, MI).

[0061] Northern blots were performed using mRNA from multiple human tissues including prostate tissue (MTN4) (obtained from Clontech, Palo Alto, CA). To determine the transcript size(s) and tissue distribution pattern of potential transcripts, MTN4 blots were hybridized with PCR products spanning the entire RNase L gene. The probes were labeled with α -³²P-dCTP by random priming (Stratagene, La Jolla, CA) following the manufacturer's directions. Hybridization was carried out at 42°C overnight in Hybrisol 1 hybridization buffer (Intergen, Purchase, NY), followed by stringent washing. Filters were subsequently subjected to autoradiography.

[0062] Laser capture microdissection was performed using glass slides containing 8 μ m thick sections cut from paraffin-embedded tissue blocks. The glass slides were deparaffinized, stained with hematoxylin and eosin, dehydrated in xylene, and then used immediately for laser capture microdissection of tumor cells using the PixCell II LCM system (Arcturus, Mountain View, CA). A polymer cap was placed on the slide and all available tumor cells (~2,000) were transferred to the cap using a laser beam. The cap was then placed on an Eppendorf tube containing 50 μ l of digestion buffer (1 mg/ml proteinase K, 10 mM Tris-HCl, 1 mM EDTA, 1% Tween-20, and pH of 8). The tubes were incubated at 52°C overnight upside down so that the digestion buffer contacted the tissue on the cap. Finally, the caps, were removed after centrifugation for 5 minutes and proteinase K was heat-inactivated by incubation at 95°C for 10 minutes.

[0063] Loss of heterozygosity (LOH) tests were performed using single-stranded conformational polymorphism (SSCP) analysis for the G275X mutation. PCR was performed to produce an expected 166 bp product spanning the G275X mutation, using forward primer - CCTGATCCTGGCAGTGGAGA and reverse primer – GGCTCCACGTTGCACAGCA. PCR reactions were modified such that volumes were reduced to 15 μ l and α -³²P-dCTP was added to the PCR reaction mix. PCR was carried out for a total of 40 cycles and then PCR products were mixed with formamide loading buffer, were denatured, and were separated by electrophoresis on a Hydrolink MDE gel (BioWhittaker Molecular Applications, Rockland, ME), using 5% glycerol for 16 hours. Gels were vacuum-dried and were subjected to autoradiography.

[0064] PTT were performed using PCR to obtain DNA (1.2 kb) containing the 5' end coding region of the RNase L gene, using forward primers PCA-MetUPT7 (GGATCCTAACGACTCACTATAGGGAGACCTGGTAGCAGG-TGGCATTACC) or PCA-MNT7 (GGATCCTAACGACTCACTATAGGG-AGACCCAGGTGGCATTACCGTCATG) and reverse primer PCA-REV2TTA (TTTTTTAGGAGGGTAAAAATCTTCTTG). Forward primers contained the T7 promoter sequence. PCR was performed on 10 ng genomic DNA, using the Advantage-HF2 PCR kit (Clontech, Palo Alto, CA) and the manufacturer's recommended protocol.

PCR products were used directly for the *in vitro* transcription-translation reaction using the TNT T7 Coupled Reticulocyte Lysate System (Promega, Fitchburg, WI) using ^{35}S -methionine and the manufacturer's recommended protocol. A 5 μl aliquot of each reaction mixture was analyzed using a NuPAGE 10% Bis-Tris gel (Invitrogen). The gel was then dried under vacuum and visualized by autoradiography.

Example 1

[0065] This example describes the association or link between the RNase L gene and HPC. This example also describes the structure of the RNase L gene, as well as the structure of the RNase L gene product. Moreover, this example describes the prevalence of the RNase L gene product in various types of tissues.

[0066] Eight high-risk prostate cancer families were selected for intensive study. These families demonstrated strong evidence of linkage between prostate cancer and the HPC1 locus and had a LOD greater than 0.70. Moreover, the eight high-risk prostate cancer families provided a series of informative affected recombinants, thus enabling a refinement of the HPC1 interval (see Figure 1B, which is a table of nucleic acid position versus pedigree). Positional cloning was performed and a 6-megabasepair (Mb) sequence ready BAC contig and transcript map of the chromosome 1 locus 1q25, which encompassed the HPC1 critical interval, was constructed. One known gene that mapped to the contig, via FISH, was the RNase L gene (OMIM#-180435) (see Figure 1C, which is a transcript map of the chromosome 1 locus 1q25). To confirm the nucleic acid position of the HPC1 gene, test samples comprising nucleic acid comprising the HPC1 gene were obtained from 91 high-risk prostate cancer families. A multi-point linkage analysis was performed on the test samples and the HPC1 gene was confirmed to be in the interval D1S2883-D1S158-D1S422 (see Figure 1A, which is a graph of nucleic acid position versus LOD).

[0067] To determine the genomic structure of the RNase L gene, the publicly available draft human genome sequence contig (GenBank accession number NT-004753) containing the entire RNase L transcription unit was analyzed (see Figure 1C). The RNase L gene consists of seven exons (see Figure 1D, which is a schematic diagram of the RNase L gene). The first exon is noncoding and contains the transcriptional start site. The initiating methionine codon is 165 nucleotides into exon 2, which is 1644 nucleotides in length (see Figure 1D). Moreover, two forms of RNase L were revealed by Northern analysis with the major 5 kb form being expressed in a number of tissues, including prostate, and a 9.5kb form, which is believed to contain a large alternative 3' untranslated region, being present primarily in the spleen and thymus. The encoded 741 amino acid RNase L protein (see Figure 1E, which is a schematic diagram of the RNase L protein) is characterized by nine ankyrin-like repeats at the amino terminus involved in protein-protein interaction, along

with two putative phosphate binding loop (p-loop) structures involved in 2'-5'A binding, which is thought to be essential for activation of RNase L. The carboxy terminus contains a tyrosine kinase-like domain and a ribonuclease domain, which is thought to be responsible for the catalytic function of the RNase L protein.

Example 2

[0068] This example demonstrates that one or more mutations can be detected in the RNase L gene in a test sample comprising a nucleic acid comprising the RNase L gene obtained from a mammal at risk for prostate cancer.

[0069] Test samples comprising the DNA of 26 patients from high-risk prostate cancer families were obtained and genomic DNA from each patient was amplified via PCR, using the M13-tailed primer sequences listed in 5'⇒3' orientation in Table 1.

TABLE 1:

RNAex1a.1f	GATTAAGTGCTAGGAGATAA	272 bp
RNAex1a.1r	CGTTATGCAGAGGTGTCCAG	
RNAex1a.2f	CGAAAGATGTTGACCTGGTCC	315bp
RNAex1a.2r	GCTTGACCTTACCATACAC	
RNAex1bf	GCGTGAAGCTGCTGAAACTT	461bp
RNAex1br	CCTCTGCACCAAACCCAAGT	
RNAex1cf	CCTGATCCTGGCAGTGGAGA	440 bp
RNAex1cr	CTTCTTGCTTCTCATAGAAC	
RNAex1df	GCTGATACTTCAGAAGGAGG	406 bp
RNAex1dr	CCACAATTCTAAGAGAGAA	
RNAex2f	GAECTCACTTTATGGAGAA	244 bp
RNAex2r	CATATGTCCTACTAGTTCTG	
RNAex3f	GGTTACCCAAATAATTAGTA	333 bp
RNAex3r	CCAGACTTCTGTCTGCACCA	
RNAex4f	GGATGATCATGATCATTAG	223 bp
RNAex4r	CACAAATTCTTATTCCCTGGA	
RNAex5f	GGTATGAATATTCATCAGG	239 bp
RNAex5r	CACATATAATTGTAGGAATG	
RNAex6f	GGCAAGCATGCTGAACAATT	293 bp
RNAex6r	CCAGAATGTTGTGATTGCC	

[0070] The genomic DNA obtained from each of the 26 high-risk patients was then cleaned, purified, and sequenced. The sequence data from each nucleic acid were aligned

and analyzed to identify nucleotide differences within the RNase L genes, using a human genome sequence containing the genomic structure of the RNase L gene as a control.

[0071] A first mutation in the RNase L gene was identified in the proband (i.e., individual 009) of the high-risk prostate cancer pedigree 065. High-risk family 065 comprises five brothers, four of which have prostate cancer and share either of two haplotypes through the HPC1 region. The pedigree structure and affected status for family pedigree 065 is detailed below in Figure 2A, which is a schematic diagram that also demonstrates a sequence-based mutational analysis and a LOH analysis for pedigree 065. Sequence analysis of all family members of pedigree 065, for which DNA was available, revealed that all four affected brothers share a guanine to thymine substitution at position 795 (i.e., 795G→T) in the second exon of the RNase L gene, starting from the initiating methionine of the RNase L gene and resulting in the conversion of a glutamic acid codon to a termination codon at amino acid position 265 (see Figure 2A). While not wishing to be held to any particular theory, it is hypothesized that this E265X mutation could result in the truncation of a significant portion of the RNase L protein, potentially leading to loss of function of that allele (see Figures 1D and 2A). In fact, such a deletion mutant of RNase L (containing only amino acids 1-265) is known to have a severely compromised ability to bind to 2',5'-oligoadenylate (i.e., about 1.9% of normal 2-5A binding activity) (see Zhou, A., Hassel, B. A. & Silverman, R. H. Expression cloning of 2-5A-dependent RNAase: a uniquely regulated mediator of interferon action. *Cell* 72, 753-65. (1993)).

[0072] A second mutation in the RNase L gene was identified in patient 023 of high-risk family pedigree 097. Patient 023 is an African American male who was diagnosed with prostate cancer at age 49. The pedigree structure and affected status for family pedigree 097 is detailed below in Figure 2B, which is a schematic that also demonstrates a sequence-based mutational analysis and a LOH analysis for pedigree 097. Sequence analysis revealed that patient 023 possessed a guanine to adenine substitution at position 3 of the second exon of the RNase L gene (i.e., 3G→A), which corresponds to the initiating methionine of the RNase L gene, and which results in the conversion of a methionine codon to an isoleucine codon at amino acid position 1 (i.e., a M1I mutation). That this methionine is the translation initiation site is evidenced by it being the first ATG in the cDNA encoding RNase L and, furthermore, by the presence of multiple stop codons lying 5' in the mRNA. Again, while not wishing to be held to any particular theory, it is hypothesized that the M1I mutation could result in a reduced translational efficiency of the RNase L gene (e.g., a reduction in translational efficiency to about 3-5% of normal levels). Moreover, the M1I mutation can potentially lead to translation initiation at a methionine codon which is downstream in the RNase L gene (e.g., a methionine codon adjacent to sequences fitting the Kozak consensus sequence), thereby leading to an out-of-frame RNase L protein; an in-

frame RNase L protein devoid of a significant number of N-terminal amino acids; and/or a lack of expressed RNase L protein (i.e., due to a null allele). Sequence analysis of all family members of pedigree 097 revealed that four of six brothers (all of which were diagnosed with prostate cancer) possessed the M1I mutation. Moreover, one brother of the 097 pedigree who possessed the M1I mutation was diagnosed with both prostate cancer and gastric cancer.

Example 3

[0073] This example demonstrates a loss of heterozygosity (LOH) of the wild-type RNase L gene in a tumor cell obtained from a mammal having at least one mutation in the RNase L gene.

[0074] To investigate allelic imbalance at the RNase L locus, LOH tests were performed using tumor cell DNA from mutation-carrying family members of pedigrees 065 and 097 (discussed previously in Example 2). Paraffin-embedded tumor cells from patients 065-09 and 097-023 were subjected to laser capture microdissection (LCM) to enrich for tumor cells. DNA was isolated from tumor cells and PCR was performed on normal and tumor DNA pairs, using primers spanning either of the G275X mutation or the M1I mutation for patients 065-009 and 097-023, respectively.

[0075] Although the tumor cell DNA of individual 023 from pedigree 097 proved problematic for amplification, a clear loss of the wild-type RNase L allele (with retention of the mutant RNase L allele) was revealed in the tumor cell DNA of individual 009 from pedigree 065 (see Figure 2A). These data indicate a complete loss of the wild-type RNase L allele and, potentially, the loss of function of RNase L in tumor cells of an HPC1-linked patient.

Example 4

[0076] This example demonstrates the production of a truncated mutant RNase L gene product in a tumor cell obtained from a mammal having at least one mutation in the RNase L gene.

[0077] To further characterize the G275X mutation in individual 009 of pedigree 065 (065-009), a PTT was performed on nucleic acids that spanned the G275X mutation. PTT utilizes *in vitro* transcription and translation to identify sequence changes in a nucleic acid which cause premature protein termination. Results from the PTT revealed the presence of a truncated peptide specific to 065-009 (Figure 2B, which is a schematic diagram that demonstrates a pedigree structure, a sequence-based mutational analysis and an LOH analysis for pedigree 097). In particular, the normal and truncated RNase L proteins were discovered to be approximately 23 kD and 15 kD, respectively (based upon molecular

weight standards), as opposed to the expected sizes of 36.7 kD and 29 kD for the normal and truncated RNase L proteins, respectively. This difference in expected versus observed sizes of the normal and truncated RNase L proteins suggests the occurrence of at least one error in the translation of the RNase L gene, wherein the translation of the RNase L gene begins at the third methionine, instead of at the first methionine, of the RNase L gene.

Example 5

[0078] This example demonstrate that the E265X mutation in the RNase L gene is present in the normal population at relatively low allelic frequency.

[0079] Analysis of DNA from several cohorts of individuals was carried out to assess the frequency of the E265X mutation (i.e., the 795G→T substitution) and M1I mutation (i.e., the 3G→A substitution) in control populations and non-HPC prostate cancer study groups (see Table 2). All individuals within the study groups of Table 2 which are designated by (*) include men aged 35-70 with prostate-specific antigen (PSA, another indicator of prostate cancer) levels below 0.70 and normal digital rectal exam (DRE) results. The test samples used for all study groups comprised blood, with the exception of the non-HPC prostate cancer study group, for which tissue test samples were used. Direct sequencing methods, SSCP, and NIaIII digests were used to screen for the E265X mutation and M1I mutation in the RNase L genes of individuals of all study groups.

TABLE 2:

Mutation	Study Groups	# Screened	# Mutant	Frequency
E265X	unaffected Caucasian men*	186	2	0.54%
	CEPH parents	96	1	0.52%
	US population controls	48	0	0.00%
		330	3	0.45%
	non-HPC prostate cancer cases *	258	2	0.39%
M1I	unaffected African-American men*	92	0	0.00%
	unaffected Caucasian men*	186	0	0.00%
	African- American population control	192	0	0.00%
	U.S. population controls	48	0	0.00%
	Total number of samples screened	240	0	0.00%
	non-HPC prostate cancer cases *	180	0	0.00%

[0080] Peripheral blood from a set of 144 normal population control individuals (96 of which are CEPH parents) revealed the presence of the E265X mutation in one individual by direct sequencing of the RNase L gene, using the methods described previously.

Subsequent analysis of 186 different unaffected men participating in a screening study for prostate cancer revealed two individuals who were E265X mutation carriers (i.e., two individuals who were heterozygous with one mutant RNase L allele and one wild-type RNase L allele). Neither of these heterozygous individuals has a family history of prostate cancer, nor did they have elevated levels of PSA. Moreover, single-stranded conformational polymorphism (SSCP) analysis of germ line DNA from 258 men with non-familial prostate cancer was performed and revealed only two individuals with the E265X mutation, both of whom were subsequently shown to be heterozygous. Thus, the E265X mutation appears to present in the general population with an allele frequency of about 0.5%.

[0081] All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

[0082] While this invention has been described with an emphasis upon preferred embodiments, variations of the preferred embodiments can be used, and it is intended that the invention can be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the claims.

WHAT IS CLAIMED IS:

1. A method of detecting cancer or a predisposition to cancer in a mammal, which method comprises detecting at least one mutation in a gene encoding interferon inducible 2',5'-oligoadenylate-dependent RNase L (RNase L) in a test sample comprising a nucleic acid comprising the RNase L gene obtained from the mammal, wherein the at least one mutation is indicative of cancer or a predisposition to cancer in the mammal.
2. The method of claim 1, wherein the cancer is prostate carcinoma.
3. The method of claim 2, wherein the prostate carcinoma is hereditary.
4. The method of claim 1, wherein the at least one mutation compromises the tumor-suppressing activity of the RNase L gene product.
5. The method of claim 1, wherein the at least one mutation compromises the ability of the RNase L gene product to bind to 2'-5'-oligoadenylate, to degrade double-stranded RNA, and/or to induce apoptosis, as compared to wild-type RNase L.
6. The method of any of claims 1-5, wherein the method comprises detecting at least one mutation in the second exon of the RNase L gene.
7. The method of any of claims 1-5, wherein the method comprises detecting at least one mutation outside of the second exon of the RNase L gene, wherein the at least one mutation affects the transcription, post-transcriptional processing, translation, and/or post-translational processing of the second exon of the RNase L gene.
8. The method of any of claims 1-5, wherein the method comprises detecting a mutation at position 795 of the second exon of the RNase L gene.
9. The method of claim 8, wherein the mutation at position 795 of the second exon is a guanine to thymine substitution.
10. The method of any of claims 1-5, wherein the method comprises detecting a mutation at position 175 of the second exon of the RNase L gene.

11. The method of claim 10, wherein the mutation at position 175 of the second exon is a guanine to adenine substitution.

12. The method of any of claims 1-5, wherein the method comprises detecting at least one mutation in the initiation codon of the RNase L gene.

13. The method of claim 12, wherein the method comprises detecting a mutation at position 3 of the second exon of the RNase L gene.

14. The method of claim 13, wherein the mutation at position 3 of the second exon is an adenine to guanine substitution.

15. The method of any of claims 1-5, wherein the method comprises detecting one or more mutations selected from the group consisting of at least one mutation in the second exon of the RNase L gene and at least one mutation outside of the second exon of the RNase L gene, wherein the at least one mutation outside of the second exon of the RNase L gene affects the transcription, post-transcriptional processing, translation, and/or post-translational processing of the second exon of the RNase L gene.

16. The method of claim 15, wherein the at least one mutation in the second exon of the RNase L gene is selected from the group consisting of a mutation at position 175 of the second exon of the RNase L gene, a mutation at position 795 of the second exon of the RNase L gene, and a mutation in the initiation codon of the RNase L gene.

17. The method of claim 16, wherein the mutation at position 175 of the second exon is a guanine to adenine substitution, the mutation at position 795 of the second exon is a guanine to thymine substitution, and the mutation in the initiation codon is at position 3 of the second exon.

18. The method of claim 17, wherein the mutation at position 3 is of the second exon is an adenine to guanine substitution.

19. A method of determining the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene in a test sample comprising a nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene obtained from a mammal, which method comprises assaying the test sample for the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene, wherein a decrease

in the level of nucleic acid comprising the wild-type RNase L gene and/or an increase in the level of nucleic acid comprising a mutant RNase L gene in the test sample as compared to a control sample is indicative of prostate cancer or a predisposition to prostate cancer in the mammal.

20. The method of claim 19, wherein the prostate carcinoma is hereditary.

21. The method of claim 19 or 20, wherein the method is used for prognosticating prostate cancer in the mammal, which method further comprises comparing the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene in the test sample to the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene, respectively, in another test sample obtained from the mammal over time, wherein a decrease in the level of nucleic acid comprising the wild-type RNase L gene and/or an increase in the level of nucleic acid comprising a mutant RNase L gene is indicative of an unfavorable prognosis, an increase in the level of the nucleic acid comprising the wild-type RNase L gene and/or a decrease in the level of the nucleic acid comprising a mutant RNase L gene is indicative of a favorable prognosis, and no change in the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene is indicative of no change in the prostate cancer.

22. The method of claim 19 or 20, wherein the method is used for assessing the efficacy of treatment of prostate cancer in the mammal with a given anti-cancer agent, which method further comprises comparing the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene in the test sample to the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene, respectively, in another test sample obtained from the mammal over time, wherein a decrease in the level of nucleic acid comprising the wild-type RNase L gene and/or an increase in the level of nucleic acid comprising a mutant RNase L gene is indicative of the anti-cancer agent being effective, an increase in the level of the nucleic acid comprising the wild-type RNase L gene and/or a decrease in the level of the nucleic acid comprising a mutant RNase L gene is indicative of the anti-cancer agent being ineffective, and no change in the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene is indicative of no change in the prostate cancer due to treatment with the anti-cancer agent.

23. A method for detecting cancer or a predisposition to cancer in a mammal, which method comprises detecting a mutant RNase L in a test sample comprising protein

comprising RNase L obtained from the mammal, wherein the presence of a mutant RNase L in the test sample is indicative of cancer or a predisposition to cancer in the mammal.

24. The method of claim 23, wherein the cancer is prostate carcinoma.

25. The method of claim 24, wherein the prostate carcinoma is hereditary.

26. The method of claim 23, wherein the tumor-suppressing activity of the mutant RNase L is compromised.

27. The method of claim 23, wherein the ability of the mutant RNase L to bind to 2'-5'-oligoadenylate, to degrade double-stranded RNA, and/or to induce apoptosis is compromised as compared to wild-type RNase L.

28. The method of any of claims 23-27, wherein the method comprises detecting a mutation at amino acid position 265 of the mutant RNase L.

29. The method of claim 28, wherein the mutation at amino acid position 265 of the mutant RNase L results in the conversion of a glutamic acid codon to a termination codon.

30. The method of any of claims 23-27, wherein the method comprises detecting a mutation at amino acid position 1 of the mutant RNase L.

31. The method of claim 30, wherein the mutation at amino acid position 1 of the mutant RNase L results in the conversion of a methionine codon to an isoleucine codon.

32. A method of determining the level of wild-type RNase L and/or a mutant RNase L in a test sample comprising protein comprising wild-type RNase L and/or a mutant RNase L obtained from a mammal, which method comprises assaying the test sample for the level of wild-type RNase L and/or a mutant RNase L, wherein a decrease in the level of wild-type RNase L and/or an increase in the level of a mutant RNase L in the test sample as compared to a control sample is indicative of prostate cancer or a predisposition to prostate cancer in the mammal.

33. The method of claim 32, wherein the prostate cancer is hereditary.

34. The method of claim 32 or 33, wherein the method is used for prognosticating a cancer in the mammal, which method further comprises comparing the level of wild-type RNase L and/or a mutant RNase L in the test sample to the level of wild-type RNase L and/or a mutant RNase L, respectively, in another test sample obtained from the mammal over time, wherein a decrease in the level of wild-type RNase L and/or an increase in the level of a mutant RNase L is indicative of an unfavorable prognosis, an increase in the level of the wild-type RNase L and/or a decrease in the level of a mutant RNase L is indicative of a favorable prognosis, and no change in the level of the wild-type RNase L and/or a mutant RNase L gene is indicative of no change in the cancer.

35. The method of claim 32 or 33, wherein the method is used for assessing the effectiveness of treatment of a cancer in the mammal, which method further comprises comparing the level of wild-type RNase L and/or a mutant RNase L in the test sample to the level of wild-type RNase L and/or a mutant RNase L, respectively, in another test sample obtained from the mammal over time, wherein a decrease in the level of the wild-type RNase L and/or an increase in the level of a mutant RNase L is indicative of the anti-cancer agent being effective, an increase in the level of the wild-type RNase L and/or a decrease in the level of a mutant RNase L is indicative of the anti-cancer agent being ineffective, and no change in the level of the wild-type RNase L and/or a mutant RNase L is indicative of no change in the cancer due to treatment with the anti-cancer agent.

36. A method of treating a mammal prophylactically or therapeutically for cancer, wherein the cancer is due to a complete or partial loss of wild-type RNase L, which method comprises providing RNase L to the mammal, whereupon the mammal is treated prophylactically or therapeutically for cancer.

37. The method of claim 36, wherein the cancer is a prostate carcinoma.

38. The method of claim 37, wherein the prostate carcinoma is hereditary.

39. The method of any of claims 36-38, wherein RNase L is provided to the mammal by administering to the mammal a nucleic acid encoding and expressing wild-type RNase L.

40. The method of any of claims 36-38, wherein RNase L is provided to the mammal by administering to the mammal the wild-type RNase L protein.

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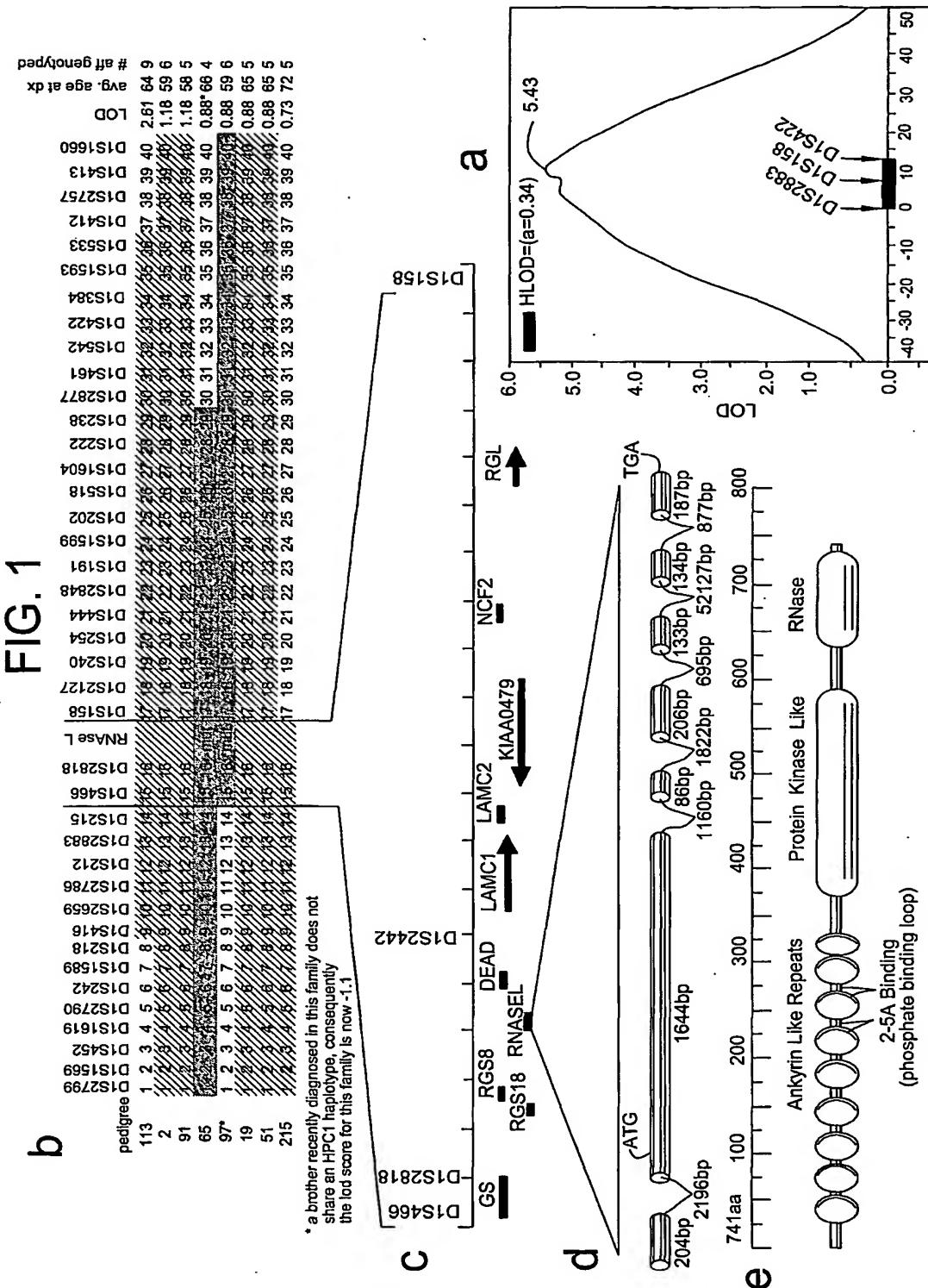
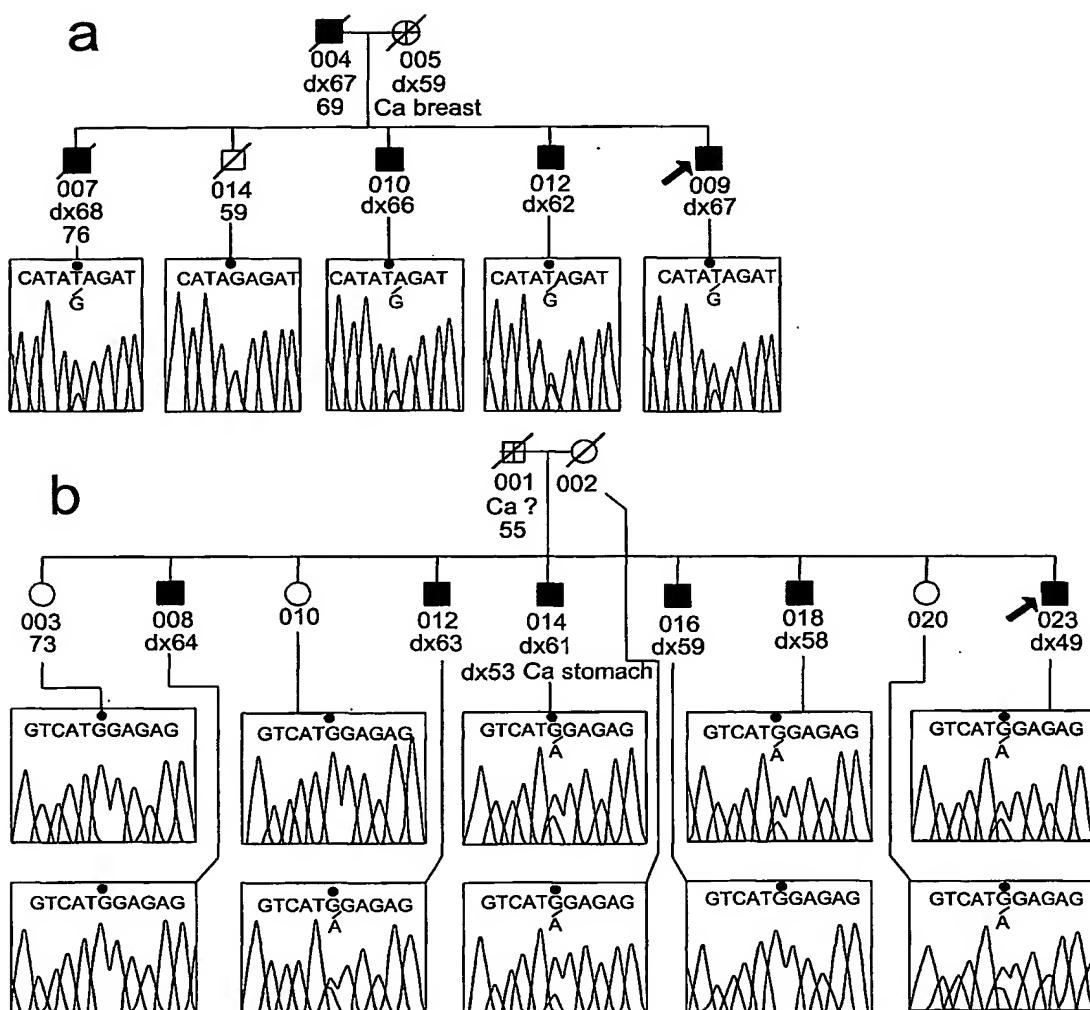


FIG. 2



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(54) Title: USE OF RIBONUCLEASE L IN DIAGNOSIS OF CANCER

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(57) Abstract: A method of detecting cancer or a predisposition to cancer in a mammal, which method comprises detecting at least one mutation in a gene encoding interferon-inducible 2',5'-oligoadenylate-dependent RNase L (RNase L) in a test sample comprising a nucleic acid comprising the RNase L gene obtained from the mammal; a method of determining the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene in a test sample comprising a nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene obtained from a mammal, which method comprises assaying the test sample for the level of nucleic acid comprising the wild-type RNase L gene and/or a mutant RNase L gene; a method for detecting cancer or a predisposition to cancer in a mammal, which method comprises detecting a mutant RNase L in a test sample comprising protein comprising RNase L obtained from the mammal; a method of determining the level of wild-type RNase L and/or a mutant RNase L in a test sample comprising protein comprising wild-type RNase L and/or a mutant RNase L obtained from a mammal, which method comprises assaying the test sample for the level of wild-type RNase L and/or a mutant RNase L; and a method of treating a mammal prophylactically or therapeutically for cancer, wherein the cancer is due to a complete or partial loss of wild-type RNase L, which method comprise providing RNase L to the mammal.

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	CARPEN et al. Germline mutations in the ribonuclease L gene in families showing linkage with HPC1. Nature Genetics. February 2002, Vol. 30, pages 181-184.	1-18
A	SIMARD et al. Perspective: Prostate Cancer Susceptibility Genes. Endocrinology. June 2002, Vol. 143, No. 6, pages 2029-2040.	19-40

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Continuation of B. FIELDS SEARCHED Item 3:
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